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SEQUENCE LISTING

<110> Miyawaki, Atsushi
Sawano, Asako

<120> METHOD FOR MUTAGENESIS

<130> 11283-012001

<140> 09/920,922

<141> 2001-08-02

<150> JP 2000-237166

<151> 2000-08-04

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<212> DNA

<213> Aequorea victoria

<220>

<221> CDS

<222> (1)...(717)

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gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc 96
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
20 25 30

gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc 144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
35 40 45

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctg gtg acc acc 192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
50 55 60

ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag 240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65 70 75 80

cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag 288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
85 90 95

cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag 336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

100	105	110	
gtg aag ttc gag ggc gac acc	ctg gtg aac cgc atc	gag ctg aag ggc	384
Val Lys Phe Glu Gly Asp Thr	Leu Val Asn Arg Ile	Glu Leu Lys Gly	
115	120	125	
atc gac ttc aag gag gac ggc	aac atc ctg ggg cac aag	ctg gag tac	432
Ile Asp Phe Lys Glu Asp Gly	Asn Ile Leu Gly His Lys	Leu Glu Tyr	
130	135	140	
aac tac aac agc cac aac gtc	tat atc atg gcc gac aag	cag aag aac	480
Asn Tyr Asn Ser His Asn Val	Tyr Ile Met Ala Asp Lys	Gln Lys Asn	
145	150	155 160	
ggc atc aag gtg aac ttc aag	atc cgc cac aac atc gag	gac ggc agc	528
Gly Ile Lys Val Asn Phe Lys	Ile Arg His Asn Ile Glu	Asp Gly Ser	
165	170	175	
gtg cag ctc gcc gac cac tac	cag cag aac acc ccc atc	ggc gac ggc	576
Val Gln Leu Ala Asp His Tyr	Gln Gln Asn Thr Pro Ile	Gly Asp Gly	
180	185	190	
ccc gtg ctg ctg ccc gac aac	cac tac ctg agc acc cag	tcc gcc ctg	624
Pro Val Leu Leu Pro Asp Asn	His Tyr Leu Ser Thr Gln	Ser Ala Leu	
195	200	205	
agc aaa gac ccc aac gag aag	cgc gat cac atg gtc ctg	ctg gag ttc	672
Ser Lys Asp Pro Asn Glu Lys	Arg Asp His Met Val Leu	Leu Glu Phe	
210	215	220	
gtg acc gcc gcc ggg atc act	ctc ggc atg gac gag ctg	tac aag	717
Val Thr Ala Ala Gly Ile Thr	Leu Gly Met Asp Glu Leu	Tyr Lys	
225	230	235	
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<212> PRT			
<213> Aequorea victoria			
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Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile			
35 40 45			
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr			
50 55 60			
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys			
65 70 75 80			
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu			
85 90 95			
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu			
100 105 110			
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly			

	115		120		125										
Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr
	130					135					140				
Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn
145					150					155					160
Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser
				165					170					175	
Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly
			180					185					190		
Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu
	195					200						205			
Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe
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gaa	tta	gat	ggt	gat	gtt	aat	ggg	cac	aaa	ttt	tct	gtc	agt	gga	gag	96
Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	
			20					25					30			
ggt	gaa	ggt	gat	gca	aca	tac	gga	aaa	ctt	acc	ctt	aaa	ttt	att	tgc	144
Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	
			35					40					45			
act	act	gga	aaa	cta	cct	gtt	cca	tgg	cca	aca	ctt	gtc	act	act	ttc	192
Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Phe	
		50				55					60					
tct	tat	ggt	gtt	caa	tgc	ttt	tca	aga	tac	cca	gat	cat	atg	aaa	cag	240
Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	
	65				70					75					80	
cat	gac	ttt	ttc	aag	agt	gcc	atg	ccc	gaa	ggt	tat	gta	cag	gaa	aga	288
His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	
				85					90					95		
act	ata	ttt	ttc	aaa	gat	gac	ggg	aac	tac	aag	aca	cgt	gct	gaa	gtc	336
Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	
			100					105					110			
aag	ttt	gaa	ggt	gat	acc	ctt	gtt	aat	aga	atc	gag	tta	aaa	ggt	att	384
Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	
		115					120					125				

gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432
 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140

tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480
 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160

atc aaa gtt aac ttc aaa att aga cac aac att' gaa gat gga agc gtt 528
 Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175

caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576
 Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190

gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624
 Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205

aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta 672
 Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220

aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa 714
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 225 230 235

taa 717

<210> 4

<211> 238

<212> PRT

<213> Aequorea victoria

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 20 25 30
 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45
 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60
 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80
 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95
 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110
 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125
 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140
 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160

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Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
      165                      170                      175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
      180                      185                      190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
      195                      200                      205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
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Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                      230                      235

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 <223> Synthetically generated primer

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26

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 <212> DNA
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21

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 <213> Aequorea victoria

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Cys	Thr		Thr	Gly	Lys	Leu	Pro	Val		Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr
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Leu	Thr		Trp	Gly	Val	Gln	Cys	Phe		Ser	Arg	Tyr	Pro	Asp	His	Met	Lys
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Gln	His		Asp	Phe	Phe	Lys	Ser	Ala		Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu
			85					90					95				
Arg	Thr		Ile	Phe	Phe	Lys	Asp	Asp		Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu
			100					105					110				
Val	Lys		Phe	Glu	Gly	Asp	Thr	Leu		Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly
			115					120					125				
Ile	Asp		Phe	Lys	Glu	Asp	Gly	Asn		Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr
			130					135					140				
Asn	Tyr		Asn	Ser	His	Asn	Val	Tyr		Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn
			145					150					155				
Gly	Ile		Lys	Val	Asn	Phe	Lys	Ile		Arg	His	Asn	Ile	Glu	Asp	Gly	Ser
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Val	Gln		Leu	Ala	Asp	His	Tyr	Gln		Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly
			180					185					190				
Pro	Val		Leu	Leu	Pro	Asp	Asn	His		Tyr	Leu	Ser	Tyr	Gln	Ser	Ala	Leu
			195					200					205				
Ser	Lys		Asp	Pro	Asn	Glu	Lys	Arg		Asp	His	Met	Val	Leu	Leu	Glu	Phe
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<210> 9

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<212> PRT

<213> Aequorea victoria

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Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile
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Cys	Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr
50						55					60				
Leu	Thr	Trp	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys
65					70					75					80
Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu
				85					90					95	
Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu
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Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly
		115					120					125			
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Gly	Ile	Lys	Ala	His	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser
				165					170					175	
Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly

			180					185				190			
Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Tyr	Gln	Ser	Ala	Leu
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Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe
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Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly	Met	Asp	Glu	Leu	Tyr	Lys	
225					230					235					